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(81) **Designated States:** AE, AL, AM, AT, AT (Utility model), AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, CZ (Utility model), DE, DE (Utility model), DK, DK (Utility model), EE, EE (Utility model), ES, FI, FI (Utility model), GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK (Utility model), SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

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(54) **Title:** LIPOSOMAL DELIVERY COMPLEX

(57) **Abstract**

A liposomal delivery complex for site–specific delivery of a pharmacological agent to a cell surface with improved targeting efficiency. The liposomal delivery complex generally comprises a liposome, an antibody, and a connecting moiety which binds the Fc region of the antibody for binding the liposome to the antibody. In a presently preferred embodiment of the invention, the connecting moiety is protein G'.

# LIPOSOMAL DELIVERY COMPLEX

## FIELD OF THE INVENTION

This invention relates generally to liposomal delivery complexes providing enhanced delivery of biologically active diagnostic and therapeutic agents. In particular, it relates to a liposomal delivery complex comprising a liposome, an antibody, and a connecting moiety which specifically binds the Fc region of the antibody to connect the antibody to the liposome.

## DESCRIPTION OF RELATED ART

Liposomes are lipid molecules formed into a typically spherically shaped arrangement defining aqueous and membranal inner compartments. Liposomes can be used to encapsulate pharmacological agents within the inner compartments, and deliver such agents to desired *in vivo* sites. However, liposomes are susceptible to removal by the body's reticuloendothelial system (RES), mainly the liver and the spleen. As part of the body's immune system, the RES will quickly phagocytize the liposome along with its cargo, thus hampering the treatment or diagnostic regime.

Lau and Geho in U.S. Patent Nos. 5,567,432 and 4,501,728, incorporated herein in their entireties by reference, describe a targeted liposomal carrier system comprising a liposome, a targeting molecule, and a RES masking molecule for preventing phagocytosis of the liposomal carrier system. The '728 patent discloses sialic acid as the RES masking molecule, and the '432 patent discloses neuraminic acid, a synthetically derived sialic acid, as the masking agent.

Liposomal carrier systems which lack site-specific binding to cell surfaces waste a portion of the pharmacological agent dosage and may produce unwanted side effects as the agent spreads to nonspecific and often undesirable areas of the body. Concurrently, the therapeutic index of the agent is decreased by non site-specific delivery.

target cell-surface antigen, but also compete for attachment to the liposomal carrier construct.

This invention completely avoids such prior art problems by providing a connecting moiety which specifically binds the Fc region of an antibody. In a presently preferred embodiment, the connecting moiety comprises a protein, known as protein G′ (i.e., "protein G prime"), which possesses binding specificity for the Fc region of antibodies. The gene for protein G from *Streptococcus* strain G 148 has been cloned and expressed in *Escherichia coli*. The regions on the gene corresponding to the albumin-binding domains and the Fab-binding region have been deleted by site-directed mutagenesis. The translation of regions corresponding to the cell-wall and membrane-binding domains has been prevented by introducing stop codons upstream of these domains. As a result, this recombinant DNA sequence encodes protein G′, which binds only the Fc portion of an antibody, Eliasson, M. *et al.* (1988) *J. Bio. Chem. 263*, pp. 4323-4327), and see, Goward, C.R., *et al* (1990) *Biochem. J.*, 267, pp. 171-177, for the nucleotide sequence and deduced amino acid sequence of Protein G′, incorporated herein in their entireties by reference.

As a result of the binding between Protein G′and the Fc region of antibodies, protein G′ shields the Fc regions of the attached antibodies from non-specific binding to cell-surfaces, other proteins, and anatomical structures. The molecular structure of protein G′ enables the molecule to accept the Fc region of an antibody without introducing chemical procedures that might denature either protein. Each protein G′ molecule can accept one or two Fc regions from monoclonal antibodies.

In another aspect of the invention, the liposomal delivery complex further includes a nonsense antibody which provides RES avoidance without competing with the targeting antibody for the intended antigen. Additionally, the native, targeting antibody molecule and nonsense antibody may be RES avoidance

antibodies, the presence of undesirable antibodies, antibody fragments and other debris are eliminated that may otherwise interfere with the binding of a targeted antibody liposomal complex to a cell-surface antigen, or the binding of the targeting antibody to the liposomal carrier construct. Additionally, in one embodiment, the liposomal delivery complex of the invention also provides for improved RES avoidance. These and other advantages of the invention will become more apparent from the following detailed description.

## BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates the nucleotide sequence and deduced amino acid sequence of protein G′, as reported in Goward, C.R., *et al* (1990), supra. Initiation of translation of the protein is shown from TTG codon starting at nucleotide position 705 to position 1260 at a stop codon, with the first 35 *N*-terminal amino acid residues of the purified protein indicated by the continuous line.

## DETAILED DESCRIPTION OF THE INVENTION

The invention generally comprises a construct for connecting an antibody or antibody fragment to a liposome, comprising a connecting moiety which specifically binds to the Fc region of the antibody and a linking moiety for connecting the connecting moiety to the liposome. Another aspect of the invention is a liposomal delivery vehicle comprising a liposome bound to a connecting moiety, and optionally including a linking moiety for connecting the connecting moiety to the liposome. The invention further comprises a liposomal delivery complex comprising a liposome, an antibody, and a connecting moiety which specifically binds to the Fc region of the antibody, used, for example, for delivering a diagnostic or therapeutic agent to a mammal.

In a presently preferred embodiment, protein G′ is the connecting molecule. However, it would be obvious to one of ordinary skill in the art that

used. Determination of the number of protein G′ molecules required would be obvious to one of ordinary skill in the art.

In one embodiment, the liposomal delivery complex of the invention is prepared by mixing an excess number of antibodies with a predetermined number of protein G′ molecules, thereby assuring saturation of all available Fc binding sites on protein G′. It is then possible, using known separation techniques, to remove the excess unbound antibodies which results in a high level of purified liposomal delivery complex. Thus, a high degree of targeting efficiency is achieved by separating the unbound antibodies from the fully functional liposomal complex. This step prevents free antibody competition for cell-surface antigens. Sepharose Cl-2B-300 gel filtration chromatography may be used to fractionate the various components of the mixture. The size of the liposomal complex that is fractionated is always large relative to the size of the debris, and this size differential enables the chromatographic separation to be effective.

A primary amino group on protein G′ reacts with the linking moiety sulfo-succinimidyl p-maleimidophenylbutyrate (sulfo-SMPB) at physiological pH to form a protein G′ p-maleimidophenylbutyrate construct. This construct expresses a maleimido functional group which is useful for creating new covalent bonds to sulfhydryl functionalities. When energy is imparted into the liposomal suspension by sonication or microfluidization, the maleimido functional group of the construct is capable of reacting with energized thiocholesterol, an anchoring component located in the liposomal membrane. This newly synthesized conjugate consisting of the liposome, p-maleimidophenylbutyrate and protein G′ can couple with the Fc region of a monoclonal antibody to produce the liposomal p-maleimidophenylbutyrate protein G′ monoclonal antibody complex.

A variety of antibodies or antibody fragments, which are preferably monoclonal antibodies, having an Fc region may be attached to the protein G′ connecting moiety of the invention. As used herein, the term antibody refers to a

to neighboring antibodies which also contain deacylated SATA moieties. Other types of cross-linking pose similar problems and make the exact or specific derivatization of an antibody at the molecular level difficult to control. For example, it is relatively simple to place a few SATA molecules on the exposed e-amino groups of lysine residues residing in the native structure of a protein. However, it is considerably more difficult to arbitrarily select the appropriate number of SATA molecules to be attached per protein molecule and very difficult to specify the derivatization of designated lysine residues on a particular protein to be reacted with SATA. Thus, because harsh chemical reactions for derivatizing the antibodies are not employed in the liposomal delivery complex of the invention, several different antibodies may be attached simultaneously to the same liposomal surface without concern for covalent or non-covalent antibody-to-antibody interaction.

Moreover, traditional procedures for the covalent attachment of antibodies usually involve employing a strong reducing agent, such as mercaptoethanol or dithiothreitol, to partially denature the protein for creating binding sites. As a result, some hydrophobic regions situated in the interior of the antibody are unintentionally exposed, and this compromises the structural integrity and targetability of the antibody. When these hydrophobic regions are exposed, the antibody becomes recognizable by the reticuloendothelial system (RES). As a consequence, the entire liposomal complex is also subject to RES recognition. These RES effects are undesirable and are counterproductive to the intended purpose of liposome targeting. Moreover, the exposed hydrophobic regions can cross-react or bind with other hydrophobic surfaces. This causes problematic associations which can lead to the aggregation or absorption of the antibodies on a variety of surfaces, and a corresponding lack of antibody targeting, which compromises the effectiveness of the delivery system.

In contrast, in the liposomal delivery complex of the invention, the association of the Fc portion of the antibody with the Fc binding portion of the

an attachment of naturally occurring sialic acid that provides RES avoidance capability. Thus, by employing the nonspecific Fc binding domain of these nonsense sialic acid-coated antibodies, direct attachment can be made to protein G′. In this way, macrophage avoidance antibodies containing sialic acid and targeting antibodies containing binding sites which attach to cell-surface antigens, can be equally or proportionately mixed as they are added to a liposomal conjugate. This use of a native antibody molecule that has no targeting capability yet possesses macrophage avoidance characteristics has been found in this invention to prolong the circulation time of targeted liposomes and thus increase the likelihood of the liposomal complex locating the designated cell-surface antigens. The interplay on the liposome surface between alternating target and macrophage avoidance antibodies can be made to function optimally and synergistically by using concentrations of antibodies that reflect the appropriate mole ratios. This new procedure provides an ease of synthesis and utility, since targeting and macrophage avoidance capability can be achieved in a single-step by the simultaneous addition of different antibodies to the surface of a liposome.

Thus, to provide a liposomal delivery complex having RES avoidance, individual protein G′ molecules are linked to the liposome, and to targeting antibodies as described above and to macrophage avoidance moieties.

When monoclonal antibodies are attached by way of the antibody Fc region, the sialic acid of the native antibody molecule functions to inhibit uptake of the entire liposomal complex by the various macrophage systems. Furthermore, it has been found that the native proteinaceous structures of the monoclonal antibody and protein G′ contain lysine and cysteine residues that provide reactive sites where additional derivatized sialic acid moieties can be covalently attached to create an enhanced macrophage avoidance system for the entire complex as well as for individual components. The new macrophage avoidance molecules are optimally oriented toward the bulk phase media and are not sterically restricted by other molecules of the liposomal complex. In one

antibodies. These antibodies exhibit the necessary cell surface antigen specificity. The flexibility offered by this integrated drug delivery system provides for the attachment of single or multiple numbers of antibody molecules and augments the potential for correct and multiple antibody cell-surface interactions.

Another aspect of this invention is the method of forming a liposomal carrier in which sonication energy is applied to a mixture of the liposomal, linking moiety, and connecting moiety components which form the liposomal carrier. In a presently preferred method of the invention, the connecting moiety comprises protein G′, and the liposome has a thiocholesterol moiety. In the method of the invention, a protein G′ construct, comprising protein G′ bonded to a linking moiety is combined with the liposome, and sonication energy, is applied to the mixture. The sonication energy breaks up the lipid structures into smaller liposome structures, typically about 200 Å to about 1500 Å in diameter. Consequently, the thiocholesterol groups, which otherwise would not be presented to the external phase media due to the hydrophobicity of the cholesterol, are exposed on the liposome surface. Thus, the availability of the thiocholesterol groups is enhanced, to thereby enhance the binding between the linking moiety and the liposome. Additionally, the sonication speeds up the reaction between the components by increasing the number of collisions therebetween.

The liposomal delivery complexes of this invention provide useful agents for pharmaceutical applications for administering an active agent to a host. Accordingly, the complexes of this invention are useful as pharmaceutical compositions in combination with pharmaceutically acceptable carriers. Administration of the complexes described herein can be via any of the accepted modes of administration for the biologically active substances that are desired to be administered. These methods include oral, topical, parenteral, ocular, transdermal, nasal and other systemic or aerosol forms.

Depending on the intended mode of administration, the compositions used may be in the form of solid, semi-solid or liquid dosage forms, such as, for

be integrated over a total time period of the sustained-release device in order to compute the appropriate dose required. Although effective dosage ranges for specific biologically active substances of interest are dependent upon a variety of factors, and are generally known to one of ordinary skill in the art, some dosage guidelines can be generally defined. For most forms of administration, the protein prime liposomal component will be suspended in an aqueous solution and generally not exceed 30% (w/v) of the total formulation. The drug component of the formulation will most likely be less than 20% (w/v) of the formulation and generally greater than 0.01% (w/v).

In general, topical formulations are prepared in gels, creams or solutions having an active ingredient in the range of from 0.001% to 10% (w/v), preferably 0.01 to 5%, and most preferably about 1% to about 5%. (Of course, these ranges are subject to variation depending upon the potency of the therapeutic agent, and could in appropriate circumstance fall within a range as broad as from 0.001% to 20%.) In all of these exemplary formulations, as will other topical formulations, the total dose given will depend upon the size of the affected area of the skin and the number of doses per day. The formulations be applied as often as necessary, but preferably not more than about three times per day.

For oral administration, a pharmaceutically acceptable, non-toxic composition is formed by the incorporation of any of the normally employed excipients, such as, for example, mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum, cellulose, sodium crosscarmellose, glucose, gelatin, sucrose, magnesium carbonate, and the like. Such compositions include solutions, suspensions, tablets, dispersible tablets, pills, capsules, powders, sustained-release formulations and the like.

Preferably the compositions will take the form of a pill or tablet. Thus the composition will contain along with the active ingredient: a diluent such as lactose, sucrose dicalcium phosphate, or the like; a lubricant such as magnesium

sufficient quantity of a pharmaceutically acceptable liquid carrier, e.g. water, to be easily measured for administration.

Alternatively, liquid or semi-solid oral formulations may be prepared by dissolving or dispersing the protein G´ liposomal complex in vegetable oils, glycols, triglycerides, propylene glycol esters (e.g., propylene carbonate) and the like. and encapsulating these solutions or suspensions in hard or soft gelatin capsule shells.

Other useful formulations include those set forth in U.S. Patents Nos. Re. 28,819 and 4,358,603.

A more recently devised approach for parenteral administration employs the implantation of a slow-release or sustained-release system, such that a constant level of dosage is maintained. See, e.g., U.S. Patent No. 3,710,795.

The percentage of active agent contained in such parenteral compositions is highly dependent on the specific nature thereof, as well as the activity of the compound and the needs of the subject. However, percentages of active ingredient of 0.01% to 10% in solution are employable, and will be higher if the composition is a solid which will be subsequently diluted to the above percentages. Preferably the composition will comprise 0.2 - 2% of the active agent in solution.

Nasal solutions of the liposomal complex along or in combination with pharmaceutically acceptable excipients can also be administered.

Formulations of the protein G´ liposomal complex may also be administered to the respiratory tract as an aerosol for a nebulizer. In such a case, the particles of the formulation have diameters of less than 50 microns, preferably less than 10 microns.

The ease of simply mixing antibodies with a liposomal protein G´ vehicle to completely form the targeted liposomal carrier complex obviates the need for elaborate chemistry and painstaking chemical procedures in the final step of the formulation. As a consequence of the simple nature of the antibody-conjugate

cis-retinoic acid, paclitaxel, docetaxel; and biologic agents, such as interferon-α, interferon-β, interferon-γ tumor necrosis factor, erythropoietin, granulocyte colony-stimulating factor, granulocyte macrophage colony-stimulating factor, macrophage colony-stimulating factor. interleukin-1, interleukin-2.

## EXAMPLE 1

Procedure for the Preparation of the Protein G´-Linking Moiety Construct of Sample Code #3177.

1. 1.0 mg of protein G´ was solubilized in 0.4 ml of phosphate buffered saline (PBS) at pH 7.5.
2. 1.1 mg of sulfo-succinimidyl-p-maleimidophenylbutyrate (sulfo-SMPB) was solubilized in 0.4 ml of PBS buffer with accompanying sonication.
3. Solutions from Steps 1 and 2 were combined and brought to a total volume of 1.0 ml with PBS buffer at pH > 7.0 but < pH 8.0.
4. The pH was checked so that it could be adjusted, if necessary, with 0.1 N NAOH in order to be in compliance with Step 3.
5. The solution from Step 3 was allowed to react for 2 hours at ambient temperature.
6. Then 1.0 ml of the reactant mixture was chromatographed over a (1.5 x 25 cm) Sephadex G-15 column equilibrated with PBS buffer pH 7.5.
7. After the free sulfo-SMPB or the accompanying hydrolytic degradation products were removed, the peak product fractions # 11, 12, 13 were collected and pooled. The resulting 4.5 ml volume was placed into a Centricon-10 centrifugal concentrator and concentrated by centrifuging at 5,000 rpms x g for 20 minutes at 10 °C using the Sovall Model RC2-B Refrigerated Centrifuge. The filtrate was then Centriconed again until a final volume of 1.0 ml was achieved.

7. 790 µl of the supernatant was chromatographed over a (1.5 x 25 cm) Sepharose CL-2B-300 column equilibrated with PBS buffer pH 7.5.

8. Following chromatography fractions # 9-14 containing the liposomal lipid were pooled and placed in a Jouan RC10.22 centrifugal evaporator and concentrated to the original 790 µl volume.

9. Norleu-10 antibody stock solution was prepared at a concentration of 0.5 mg/23 µl. This solution was then diluted to a total volume of 250 µl with PBS buffer pH 7.5.

10. Then 250 µl of the lipid concentrate from Step 8 was mixed slowly with 120 µl of antibody solution from Step 9 and allowed to react for two hours at ambient temperature.

11. The liposomal suspension was then chromatographed over a (1.5 x 25 cm) Sepharose Cl-2B-300 column equilibrated with PBS buffer pH 7.5 to remove any free or unbound antibody.

12. Following chromatography fraction #11, which contained 1.5 ml of the liposomal protein G´ antibody complex, was determined to have the highest lipid concentration and was used as the incubation media in the LS-18 colon cancer cell culture binding study.

## EXAMPLE 3

Binding of Liposomal Delivery Complex to Colon Cancer Cells.

Initially, the LS-180 colon cancer cell culture study protocol provided for 1 x $10^{+5}$ LS-180 cells to be plated per sample well. After a suitable cell growth phase, the study was started and it was determined that there were 3.3 x $10^{+11}$ cells per sample well. Into each experimental well 19.24 x $10^{+10}$ liposomes were introduced that averaged 1980 Å in diameter. The number of liposomes was determined from a previous calculation that yielded 5.97 x $10^{-19}$ moles of lipid per liposome. The experimental sample well also contained 1.09 µg of monoclonal

**What Is Claimed Is**:

1. A construct for connecting an antibody or antibody fragment to a liposome, comprising protein G′ (SEQ ID NO: 2) and a linking moiety for connecting the protein G′ to the liposome.

2. The construct of claim 1 wherein the construct specifically binds the Fc region of the antibody.

3. The construct of claim 1 wherein the linking moiety is selected from the group consisting of sulfo-succinimidyl p-maleimidophenylbutyrate (sulfo-SMPB), p-maleimidophenylbutyrate phosphatidylethanolamine (MPB-PE), 2-iminothiolane, and succinimidylacetylthio acetic acid (SATA).

4. A liposomal delivery vehicle, comprising:

a) a liposome; and

b) a connecting moiety connected to the liposome, which specifically binds the Fc region of an antibody, for connecting the antibody to the liposome.

5. The liposomal delivery vehicle of claim 4 wherein the connecting moiety comprises protein G′(SEQ ID NO: 2).

6. The liposomal delivery vehicle of claim 4 further including a linking moiety for connecting the connecting moiety to the liposome.

7. The liposomal delivery vehicle of claim 6 wherein the linking moiety is selected from the group consisting of sulfo-succinimidyl p-maleimidophenylbutyrate (sulfo-SMPB), p-maleimidophenylbutyrate phosphatidylethanolamine (MPB-PE), 2-iminothiolane, and succinimidylacetylthio acetic acid (SATA).

8. The liposomal delivery vehicle of claim 4 further including an antibody or antibody fragment connected to the liposome.

9. The liposomal delivery vehicle of claim 4 wherein the antibody is a monoclonal antibody.

20. A liposomal delivery complex, comprising:

a) a liposome;

b) a targeting antibody connected to the liposome, which binds to cell surface antigens associated with a targeted situs; and

c) a connecting moiety having an Fc receptor bound to or in intimate association with an Fc region of the targeting antibody, for connecting the antibody to the liposome.

21. The liposomal delivery complex of claim 20 further comprising sialic acid groups bound to the targeting antibody.

22. The liposomal delivery complex of claim 20 further comprising a nonsense antibody bound to or in intimate association with the Fc receptor of the connecting moiety, wherein the nonsense antibody does not bind to a receptor associated with the targeted situs.

23. The liposomal delivery complex of claim 22 further comprising sialic acid groups bound to the nonsense antibody.

24. The liposomal delivery complex of claim 23 further comprising a diagnostic or therapeutic agent entrapped within or associated with said liposome.

25. The liposomal delivery complex of claim 24 wherein the diagnostic or therapeutic agent is selected from the group consisting of antibiotics, antidepressants, antitumorigenics, antivirals, cytokines, hormones, imaging agents, neurotransmitters, and stimulants.

26. A liposomal delivery complex for delivering antimetabolites to a targeted situs in a mammal, comprising:

a) a liposome;

b) an antibody or antibody fragment connected to the liposome, which binds to cell surface antigens associated with the targeted situs;

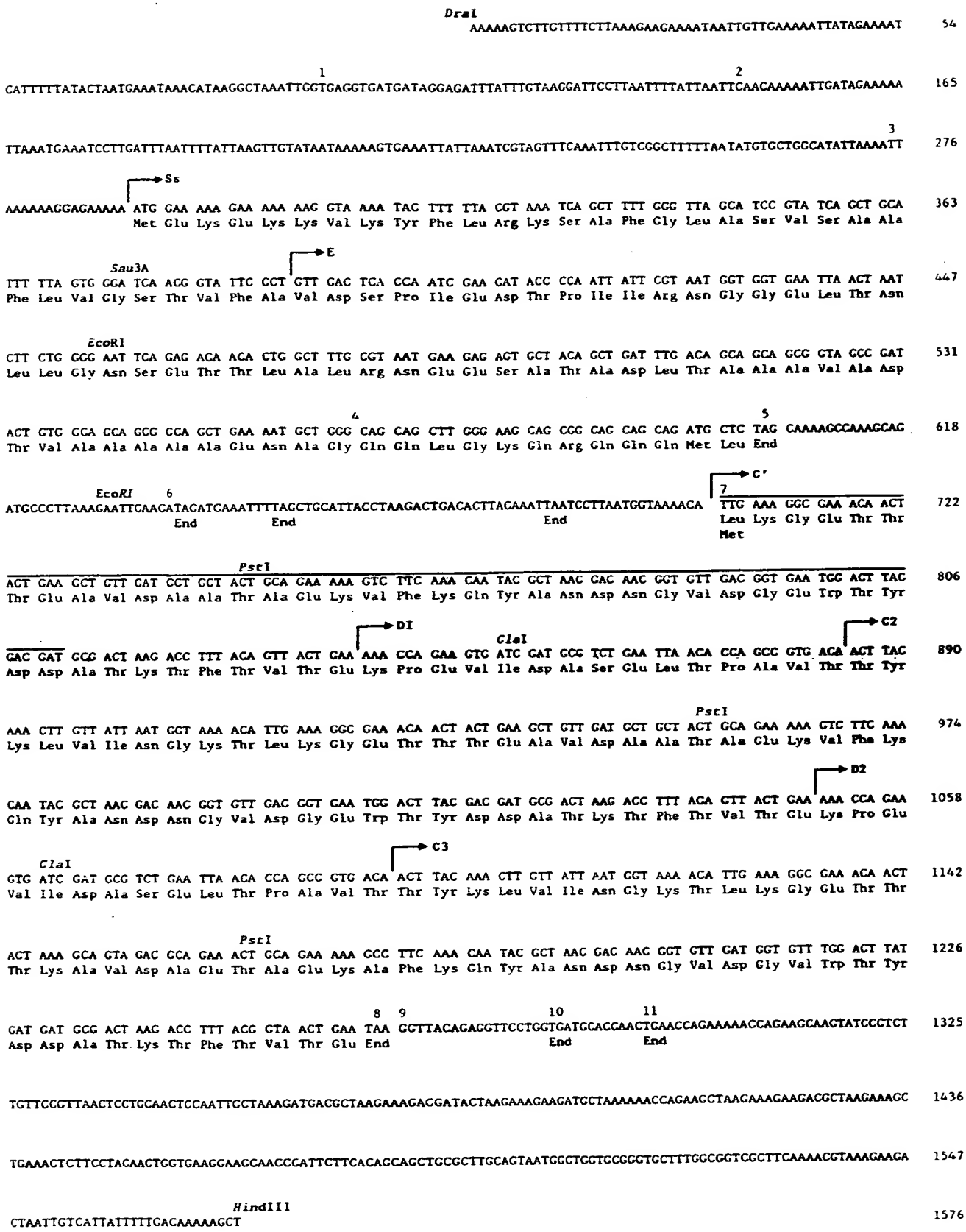


FIGURE 1

SUBSTITUTE SHEET (Rule 26)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

TTGAAAGGCG AAACAACTAC TGAAGCTGTT GATGCTGCTA CTGCAGAAAA AGTCTTCAAA 60

CAATACGCTA ACGACAACGG TGTTGACGGT GAATGGACTT ACGACGATGC GACTAAGACC 120

TTTACAGTTA CTGAAAAACC AGAAGTGATC GATGCGTCTG AATTAACACC AGCCGTGACA 180

ACTTACAAAC TTGTTATTAA TGGTAAAACA TTGAAAGGCG AAACAACTAC TGAAGCTGTT 240

GATGCTGCTA CTGCAGAAAA AGTCTTCAAA CAATACGCTA ACGACAACGG TGTTGACGGT 300

GAATGGACTT ACGACGATGC GACTAAGACC TTTACAGTTA CTGAAAAACC AGAAGTGATC 360

GATGCGTCTG AATTAACACC AGCCGTGACA ACTTACAAAC TTGTTATTAA TGGTAAAACA 420

TTGAAAGGCG AAACAACTAC TAAAGCAGTA GACGCAGAAA CTGCAGAAAA AGCCTTCAAA 480

CAATACGCTA ACGACAACGG TGTTGATGGT GTTTGGACTT ATGATGATGC GACTAAGACC 540

TTTACGGTAA CTGAATAA 558

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(72) Inventor: LAU, John, R.; 585 King Beach Drive, Howard, OH 43028 (US).

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(81) Designated States: AE, AL, AM, AT, AT (Utility model), AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, CZ (Utility model), DE, DE (Utility model), DK, DK (Utility model), EE, EE (Utility model), ES, FI, FI (Utility model), GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK (Utility model), SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

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(54) Title: LIPOSOMAL DELIVERY COMPLEX

(57) Abstract

A liposomal delivery complex for site–specific delivery of a pharmacological agent to a cell surface with improved targeting efficiency. The liposomal delivery complex generally comprises a liposome, an antibody, and a connecting moiety which binds the Fc region of the antibody for binding the liposome to the antibody. In a presently preferred embodiment of the invention, the connecting moiety is protein G'.

# INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/US 99/11177

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K47/48

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| E | WO 99 59545 A (SDG INC)<br>25 November 1999 (1999-11-25)<br>claims | 1-30 |
| A | WO 97 39735 A (SDG INC)<br>30 October 1997 (1997-10-30)<br>claims | 1-30 |
| P,X | WO 99 01110 A (SDG INC)<br>14 January 1999 (1999-01-14)<br>claims | 1 |
| A | US 5 567 432 A (LAU JOHN R ET AL)<br>22 October 1996 (1996-10-22)<br>cited in the application<br>claims | 1-30 |

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

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"O" document referring to an oral disclosure, use, exhibition or other means

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| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 22 December 1999 | 01/02/2000 |

| Name and mailing address of the ISA | Authorized officer |
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| European Patent Office, P.B. 5818 Patentlaan 2<br>NL – 2280 HV Rijswijk<br>Tel. (+31–70) 340–2040, Tx. 31 651 epo nl,<br>Fax: (+31–70) 340–3016 | Berte, M |

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Information on patent family members

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| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
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